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PLANT PRODUCTS OF ECONOMIC POTENTIAL IN HAWAII. I. BIXIN.

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INTRODUCTION

An amendment to the color additives section of the U. S. Pure Food Act passed by the last Congress specifies the conditions of safety an additive must meet before being used in or on a product that comes in contact with the mouth. The "Delaney" amendment prohibits the use of any material which has been shown to produce cancer "in animals or man, no matter how wide the difference between the amounts that could cause cancer and the amount which might be ingested through the use of the substance in a color additive". The amendment will affect particularly the coal-tar dyes which are used to a substantial degree as the red and yellow coloring agents in some food products and cosmetics. It is expected that the amendment will greatly stimulate the search for new red and yellow dyes and markedly increase the market for the known dyes of plant or animal origin, which are, in general, acceptable under the new law.

This action was anticipated and investigations were begun in a small way in 1958 to evaluate known natural coloring agents growing in Hawaii. One of the best known and most widespread is bixin, which is derived from the seed coat of *Bixa orellana*, the Lipstick Pod plant. Bixin is not considered to be carcinogenic (5).

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THE PLANT AND ITS USES

The Lipstick Pod plant or Annatto (Arnotto) plant has been used in Hawaii for many years as an unusual ornamental shrub, especially in dry arrangements. The major use for the plant on a world-wide basis, however, is for the annatto dye, a yellow to red coloring material produced from the seeds. It is used locally as a food coloring source occasionally and to a substantial extent nationally as the coloring material in butter, cheese, and margarine. Bixin is now used only rarely as a textile dye since the color is fugitive to light but it was formerly used as a cotton dye as well as for silk and wool. It was found to resist the action of soap and dilute acids very well. For printing purposes the dye is dissolved in caustic soda and developed with acid, alum, or stannous chloride. It has been used for coloring oils, soap, and woodstains. In the Philippine Islands a brown shoe polish is made from the seeds (*2, 8*) and a floor wax is made by dissolving the dye in kerosene. It has been used as a medicinal herb reportedly effective for fevers and colds and the red resinous substance is considered a remedy in some places for certain skin diseases. Its original use was as a war paint by South American Indians.

A fairly good fiber may be obtained from the bark of the plant which also contains low amounts of the dye.

Bixa orellana is a small tree, 10 to 20 feet in height at maturity. The species is monotypic in the family *Bixaceae* and is native to tropical America but is now found in all tropical countries where it occurs in moist habitats (river banks) (*7*). The leaves are more or less heart-shaped (fig. 1) with a broad base and pointed apex. They are glabrous and from 6 to 8 inches long and 3 to 5 inches wide. The flowers are white to pink or light red, 2 to 4 inches in diameter, and are borne in conspicuous clusters. The capsules are ovoid or rounded, green or brown to reddish-purple, covered with soft prickles (a variety with glabrous capsules is recorded from Liberia), and about 1 to 1½ inches long. The dark red seeds number 10 to 50 per capsule and are contained in a parchment-like inner capsule. The stamens are inserted on a hypogynous or perigynous disc. The embryo is included in the axis of the fleshy endosperm (*3*).

The plant is fairly common in tropical America and the Caribbean but is also known to grow in Malaya, India, Indonesia, Philippine Islands, Bengal, Ceylon, Nigeria, Togo, Gold Coast, Kenya, Tanganyika, Sierra Leon, Zanzibar, Belgian Congo, and Liberia. Most of the U. S. supply comes from the Caribbean area where it is produced by small landholders and from Cayenne, Brazil, India, and from the New Orleans area in Louisiana.

Figure 1. *Bixa orellana*, the Lipstick Pod plant, source of bixin.
Upper, Aspect, growing at Waimanalo. Lower, Immature pod, pointed variety.

THE DYE AND ITS PRODUCTION

The commercial annatto dye is marketed as cakes containing 6 to 12 percent dye and not more than 5 percent ash. The cakes are prepared by macerating the seeds and pulp in water, filtering off the annatto which separates out, and drying the filtered product in the sun. Strictly speaking, "annatto" is the fermented product but it is rarely produced in that fashion any more since it was found that fermentation reduced the dye content. "Cayenne" is the most desirable natural product containing 10 to 12 percent of the dye while the Brazilian type may yield less than 6 percent of bixin. The Colour Index (*1*) lists annatto as Natural Orange 4 or C. I. 75120. It contains principally bixin ($C_{25}H_{30}O_4$), a monomethyl ester of an unsaturated dicarboxylic acid (*9*), but at least six other coloring materials are present, including zeta-carotene (*4*). In purified state it forms a red crystalline dye. The fastness of the dye is rated as poor in light, but good in dilute acids and chlorine, and after washing and milling.

Yields in India at the turn of the century (*12*) were reported to be about 550 pounds of seed yielding 100 pounds of commercial dye material per acre.

TESTS IN HAWAII

Two major varieties occur in the Islands: one with a round pod and the standard, pointed-pod variety. Seeds of each were collected and planted in rows 6 feet apart and 80 feet long, at the Waimanalo Farm of the Hawaii Agricultural Experiment Station in October, 1958. *Bixa* seeds from the island of Hawaii were found to have a relatively low germination for either variety as were those from Oahu. Neal (*11*) reports that the plant grows well from seed but that propagation from cuttings is unsuccessful. Germinations were 13 and 20 percent, respectively, for the round and pointed-pod varieties.

The plants at Waimanalo grew vigorously except in the dry summer months, and harvests were begun 18 months after planting. The plant normally flowers twice a year, in May or June and in October or November. The first harvests were made in May, 1960. Harvested pods were collected, air-dried, and shelled by hand.

The whole seed was analyzed for bixin content by a modification of the method of Meyer and de Vos (*10*). One gram of whole seed was placed in a 250 ml Erlenmeyer flask (with ground glass stopper) with 50 ml of pyridine and 10 grams of quartz sand and mechanically shaken for 1 hour. The liquid was then transferred to a 100 ml volumetric flask through glass wool placed in a funnel. The solids were washed with pyridine.

An aliquot of the pyridine solution was dissolved in 70 percent ethanol and the absorbance read on a Beckman Model B Spectrophotometer. The bixin content was calculated using an $E^{1\%}_{1cm}$ of 3200 at 455 mμ (*6*).

The seeds were separated from the sand, air-dried, and weighed. The weight of the base-soluble material was calculated by difference.

Table 1. Yields of seed of *Bixa orellana* and annatto dyestuff from plantings at Waimanalo, Oahu, 1960-61.

Harvest date	YIELD				
	Whole seed (air-dry)	Base-sol. material	Bixin in base-sol. material	Bixin in seed	Commercial annatto (10% basis, cumulative)
	grams	%	%	%	lbs/acre
		Round-pod variety			
May	4263	7.9	37	2.9	124
September	191	6.7	37	2.5	128
January	12966	8.4	30	2.4	439
		Pointed-pod variety			
May	4459	9.5	42	4.0	178
August	973	8.5	38	3.2	209
September	623	7.4	49	3.6	232
December	3470	9.0	44	4.0	371
January	13875	9.1	48	4.1	939

The results of harvests and analyses for the first crop year are presented in table 1. The marked seasonal peak in the winter months and the substantial difference between the two varieties in dyestuff yield are apparent. The pointed-pod variety outyielded the round-pod variety by 500 pounds per acre or by about 115 percent. The difference appears to be brought about by a combination of superior seed yield and a greater yield of bixin in the more plentiful base-soluble material (testa).

Karrer and Jucker (*9*), in their description of the isolation of bixin from *Bixa* seed, report 100 kg of seed to yield about 5–6 kg of material which contains 15–30 percent bixin. The 5–6 percent of "material" is the fraction comparable to the 6.7 to 9.5 percent base-soluble material in table 1. The 37–49 percent bixin in the base-soluble fraction is considerably higher than the 15–30 percent of Karrer and Jucker but the method of separation and analysis was not identical with theirs.

Differences in seed yield appear to be caused by differences in the flowering potential in favor of the pointed-pod variety. The number of pods produced per plant is strikingly different but the yield of seed per pod is almost exactly the same for both varieties, being about 0.8 gm per pod.

The residual seed material was subjected to a proximate analysis in order to get a preliminary estimate of the feeding value of the by-product. It appears (table 2) that the seed residue is of high quality as a feedstuff and warrants further testing in experimental rations.

Table 2. Proximate analysis of residual seed material of *Bixa orellana* after extraction of bixin

	Lot I (%)	Lot II (%)	Mean (%)
Protein	12.5	12.5	12.5
Ether extract*	6.1	5.7	5.9
Fiber	9.8	12.9	11.4
Ash	5.2	4.3	4.8

*Residual dye material on the seed is in this fraction.

FUTURE CONSIDERATIONS

Several problems are still to be solved before *Bixa* can be recommended as a commercial crop.

1. Row-spacing, cultural, and fertility requirements may be investigated if the current tests indicate additional evaluation as a row-crop is warranted.

2. Harvesting methods must be mechanized before it can become a commercial crop in the U. S. It is assumed that a harvester of the cotton-picker type could be devised that would remove ripe pods and effectively separate the seeds. Severe pruning at the time of harvest does not seriously harm the plant and would be necessary to keep growth within reach of most harvesting devices presently known.

3. Processing would need to be mechanized but is simple and probably could easily be effected.

Some general criteria used in the evaluation of potential economic crops are satisfied by *Bixa*.

1. It is well adapted to an environmental niche in Hawaii, growing in the wet lowlands in rocky soils.

2. It is easy to establish and cultivate.

3. It is apparently free of serious pests and diseases.

4. The final or processed product is a high-value, low-volume one that might be profitably exported.

5. There is a good possibility for mechanization of culture, harvest, and processing.

6. The possibility exists for a high and rising demand and price.

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